## REMARKS

### A. Interview Summary

A telephone interview was held on March 12, 2004 between Applicants' representative and Examiner Spiegler. The finality of the Office Action dated February 25, 2004 was discussed. The Office Action was made final on the grounds that Applicants' amendment necessitated new rejections. Applicants' representative argued that the Office Action should not have been made final because the amendment merely incorporated the limitation of previously examined claim 10 into the independent claims. Examiner Spiegler found the argument persuasive, and withdrew the finality of the Office Action.

### B. Status of the Claims

Claims 1-7, 9, 11-19, and 21-57 are pending in this application. Claims 49-52 have been canceled without prejudice or disclaimer in view of the restriction requirement issued June 26, 2002. Claims 1, 6, 7, 9, 13, 16-18, and 57 have been amended to delete the recitation "dideoxynucleotide derivative." Additionally, claim 1 has been amended to incorporate the limitations of dependent claims 2 and 3. Claims 2 and 3 have been canceled. Claims 4, 6, and 7 have been amended to depend from claim 1. Claim 9 has been amended to incorporate the limitation of dependent claim 11. Claim 11 has been canceled. Claims 30 and 31 have been amended to correct the antecedent basis.

Thus, claims 1, 4-7, 9, 12-19, 21-48, and 53-57 are currently pending. The specific grounds for rejection, and Applicants' response, are set out in detail below.

## C. The Rejections Under 35 U.S.C. § 102 Are Overcome

### *1. Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 Are Not Anticipated by Short*

Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 were rejected under § 102(b) as anticipated by Short (WO 98/01581). Applicants respectfully traverse.

To anticipate a claim, the reference must teach every element of the claim (MPEP § 2131). Short does not teach every element of Applicants' claimed invention.

#### *a) The Short Reference Does Not Teach Defined Primers*

A defined primer, as used in the present invention, is a primer that generates a pool of extension products that share a defined 5' end. This is described in the specification at page 38, line 23 to page 39, line 7, and FIG. 1. In contrast, the primers described by Short result in products with undefined 5' ends.

Short specifically teaches random primers, and all of its examples appear to be based on random primers. The obvious result of random primer extension is the generation of a population of extension products with random 5' and 3' ends. The Action asserts that oligonucleotides that anneal to a population of random extension products act as defined primers because they anneal to a defined region. However, these are not "defined primers" as described in the present specification because they do not generate a pool of extension products that share a defined 5' end.

#### *b) The Short Reference Does Not Teach a Second Template Nucleic Acid Whose Sequence is Not Identical to the First Template Nucleic Acid*

Step (d) of present claims 1 and 9 recites "annealing the extended nucleic acid to at least a second single stranded template nucleic acid whose sequence is ***not identical*** to the first

template nucleic acid..." The Examiner has failed to show where Short teaches this limitation. The pages from Short cited in the action (p. 7-9 and 67) do not appear to disclose this limitation.

*c) The Short Reference Does Not Teach Adding At Least One Chain-Terminating Agent, Wherein the Chain-Terminating Agent is Incorporated Into the Extended Nucleic Acid*

Short does not teach adding at least one dideoxynucleotide or dideoxynucleotide analog before or during at least one of the first extension or second extension, wherein said dideoxynucleotide or dideoxynucleotide analog is enzymatically incorporated into said extended nucleic acid.

Short defines the recitation "means for slowing or halting the PCR amplification process" on page 25. This definition does not include dideoxynucleotides or dideoxynucleotide analogs. In finding that Short teaches "dideoxynucleotide analogs," the Action has adopted an inappropriately broad definition of the term. The present specification defines an "anolog" as "a molecule that may or may not structurally resemble a naturally occurring molecule, but functions similarly to the naturally occurring molecule." Specification, page 23, lines 7-8. The Action characterizes a dideoxynucleotide analog as anything that functions to block or interrupt amplification. This is an overbroad definition that would include such things as UV light and temperature as dideoxynucleotide analogs.

A dideoxynucleotide, and thus a dideoxynucleotide analog, functions to terminate chain elongation by being incorporated into the nascent strand and then preventing the further incorporation of nucleotides because it lacks a 3' OH group. While Short may teach nucleotide analogs (*e.g.*, 5-bromouracil), it does not teach dideoxynucleotide analogs. Specifically, 5-bromouracil is not a dideoxynucleotide analog, as defined by the specification, because its incorporation into a nucleic acid sequence during extension does not terminate chain elongation.

The present specification defines a chain terminating agent as an agent that terminates chain elongation upon incorporation into an elongated chain, see page 34, lines 24-27. The only use of the term "chain terminators" in Short appears to be in claims 1-3, on pages 67-68. Short does not appear to provide any discussion or definition regarding "chain terminators."

Furthermore, it would appear that the methods described by Short in claims 1-3 would not work if Short's use of the term "chain terminators" was construed to mean a dideoxynucleotide or dideoxynucleotide analog. Specifically, subjecting the resultant polynucleotides to a second amplification would not be possible if the first amplification had been terminated with a dideoxynucleotide or dideoxynucleotide analog unless the polynucleotides 3' OH was first restored. The Action failed to show where Short teaches this essential element. Claim 5 in Short recites heating the polynucleotides to remove DNA adducts, however, heating would not remove dideoxynucleotides.

*d) Short Is Not Enabling*

Even if the claimed invention is disclosed in a printed publication, that disclosure will not suffice as prior art if it is not enabling. *In re Donohue*, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985). Applicants have shown that Short does not teach all of the elements of the claims. However, even assuming that all of the elements were present in Short, that reference still fails to provide an enabling disclosure of Applicants' claimed invention. In particular, Short does not provide any guidance concerning defined primers, incorporating and then removing dideoxynucleotides or dideoxynucleotide analogs, or using a second template not identical to the first template that would enable one of ordinary skill in the art to make and use Applicants' claimed invention.

*e) Arguments Regarding Specific Claims*

*(1) Claims 1-7, 13, and 16*

Claims 1-7, 13, and 16 recite the addition of at least one dideoxynucleotide or dideoxynucleotide analog before or during two or more of the extensions. For example, claim 13 recites "The method of claim 12, further comprising adding at least one dideoxynucleotide or dideoxynucleotide analog before or during the third extension." The Examiner has failed to show where Short teaches such a method. The Examiner notes that Short teaches that steps c) – e) on page 7 can occur a plurality of times. However, these steps do not include the blocking the amplification process. Thus, the Examiner has not established a *prima facie* case that Short anticipates claims 1-7, 13, and 16.

*(2) Claim 31*

The Examiner failed to show where Short teaches a defined first primer nucleic acid that is resistant to cleavage or exonuclease digestion. Thus, the burden of establishing a *prima facie* case of anticipation has not been met.

*f) Conclusion*

To anticipate a claim, the reference must teach every element of the claim (MPEP § 2131). For the reasons described above, Short does not teach each and every element set forth in the claims. The Action has thus failed to meet the burden under 35 U.S.C. §102. Applicants respectfully request the reconsideration and withdrawal of the rejection.

***2. Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46-47, 53-55, and 57 Are Not Anticipated by Volkov***

Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46-47, 53-55, and 57 were rejected under § 102(e) as anticipated by Volkov (USPN 6,534,292). Applicants respectfully traverse.

Volkov does not teach all of the elements of the claims. For example, Volkov does not teach performing a second extension by ***extending the extended nucleic acid*** employing the second template nucleic acid to form a twice extended nucleic acid. In Volkov, the "non-extendable fragments act only as templates, rather than as templates and primers" (Volkov Abstract). The Action failed to establish where Volkov teaches extending the extended nucleic acid. In addition, the Action failed to show where Volkov teaches a "defined primer nucleic acid."

Furthermore, Volkov does not teach removing the dideoxynucleotide or dideoxynucleotide analog from the extended nucleic acid, if a further extension is to be performed. The Action cites Volkov at column 5, lines 3-6 and 14-18 as teaching removal of nucleotides with exonucleases and other treatments. Volkov's disclosure is in a different context from Applicants' claimed method. Volkov recites, "In one embodiment, double-stranded sequences used herein may be ***denatured***. Generation of single-strand DNAs can be done in a variety of ways, including, but not limited to thermal denaturation, alkaline treatment or exonuclease treatment." Volkov, col. 5, lines 3-7 (emphasis added). The Action failed to show where Volkov teaches removing a dideoxynucleotide or dideoxynucleotide analog from an extended nucleic acid, if a further extension is to be performed.

Moreover, Volkov is not 102(e) prior art. Attached as Exhibit A to this paper is the Rule 131 Declaration of George L. Murphy, Robert A. Setterquist, and Andrew D. Ellington. The declaration establishes that the inventors conceived of the claimed invention prior to the May 8, 2000 priority date of the Volkov patent, and were diligent in reducing the invention to practice up to and including the filing of the present application. Included with the declaration are pages describing all technological aspects of the invention from an invention disclosure made

following Ambion, Inc. standard procedures prior to May 8, 2000. The invention disclosure describes, for example, annealing a defined primer nucleic acid to a first single stranded nucleic acid and performing a first extension wherein a dideoxynucleotide is incorporated into the extension product (see Step 5). Step 6 describes removing the dideoxynucleotide from the extended nucleic acid. Step 7 describes denaturing the extended nucleic acid from the first template nucleic acid, annealing the extended nucleic acid to a second template nucleic acid whose sequence is not identical to the first template nucleic acid, and performing a second extension wherein a dideoxynucleotide is incorporated into the twice extended nucleic acid. Step 7 also provides that Steps 6 and 7 can be repeated a desired number of times.

In view of the foregoing, Applicants request the withdrawal of the rejection.

### *3. Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 Are Not Anticipated by Stemmer*

Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 were rejected under § 102(e) as anticipated by Stemmer (USPN 6,506,603). Applicants respectfully traverse.

To anticipate a claim, the reference must teach every element of the claim (MPEP § 2131). It is well settled that the burden of establishing a *prima facie* case of anticipation resides with the Examiner and only if that burden is met, does the burden of going forward shift to the applicant. See *In re Sun*, 31 U.S.P.Q.2d 1451 (Fed. Cir. 1993). The Examiner failed to establish that Stemmer teaches every element of Applicants' claimed invention.

The Action merely states that Stemmer teaches Applicants' claimed method and then refers broadly to columns 4, 6, 22-23, 25-31, and 123-130 of Stemmer in support of the rejection. The Action did not specify where in these 19 columns of text an anticipating disclosure could be found. Moreover, Applicants' review of Stemmer did not reveal an anticipating disclosure.

Stemmer teaches "gene shuffling," a technique described in the background section of Applicants' specification on page 5, lines 12-29. Gene shuffling involves the ***random*** fragmentation of nucleic acid sequences, which are then reassembled by PCR to create a mixed population of nucleic acid molecules. Columns 22-23 of Stemmer, which are cited in the Action, describe this process. It is unclear which, if any, element of Applicants' claimed invention is described in columns 22-23, as alleged in the Action. If there is such an anticipating disclosure in these columns, Applicants request that the Examiner identify it more precisely by line number.

Likewise, columns 4 and 6 of Stemmer describe gene shuffling. It is unclear which, if any, element of Applicants' claimed invention is described in columns 4 and 6, as alleged in the Action. If there is such an anticipating disclosure in these columns, Applicants request that the Examiner identify it more precisely by line number.

Columns 25-31 cover a wide range of topics that are not relevant to the anticipation of the present claims. It is unclear which, if any, element of Applicants' claimed invention is described in columns 25-31, as alleged in the Action. Applicants note that there does not appear to be any disclosure in columns 25-31 of elements such as defined primers, adding at least one dideoxynucleotide or dideoxynucleotide analog, or modifying or removing the dideoxynucleotide or dideoxynucleotide analog from the extended nucleic acid if a further extension is to be performed.

Finally, the claims in Stemmer (see col. 123-130) also fail to teach every element of Applicants' claimed invention. The Action failed to establish where Stemmer teaches, for example, defined primers, annealing the extended nucleic acid to a second single stranded template whose sequence is not identical to the first template nucleic acid, adding a

dideoxynucleotide or dideoxynucleotide analog, or modifying or removing the dideoxynucleotide or dideoxynucleotide analog.

The Action states that Stemmer teaches the uses of nucleotide analogs, which function similarly to dideoxynucleotides. Even assuming that nucleotide analogs encompass dideoxynucleotides, the Action failed to show where Stemmer teaches the essential step of removing or modifying the dideoxynucleotide prior to further extension. Stemmer's method would not appear to work as recited in the claims (see *e.g.*, col. 123-124 of that patent) if a dideoxynucleotide or dideoxynucleotide analog was used to cause the incomplete extension of the polynucleotides.

The Action states that Stemmer teaches in column 9 the removal of nucleotides using polymerases having exonuclease activity. However, Stemmer's use of polymerases having exonuclease activity is not in the same context as the present invention. Stemmer appears to teach the use of an enzyme having exonuclease activity to remove non-templated nucleotides introduced at the 3' ends of product nucleotides in shuffling amplification reactions catalyzed by a non-proofreading polymerase. In other words, it appears that Stemmer is teaching the use of an enzyme with exonuclease activity during amplification to increase the fidelity of reactions catalyzed by non-proofreading polymerases.

There are additional elements of the various dependent claims that the Examiner has failed to establish as being taught by Stemmer. However, Applicants believe that the current arguments are more than sufficient to clarify that anticipation has not been established.

In view of the above, Applicants request the withdrawal of the rejection.

## D. The Rejections Under 35 U.S.C. § 103(a) Are Overcome

### *1. The Legal Standard for Obviousness*

In order to establish a *prima facie* case of obviousness, three basic criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP § 2142.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q. 2d 1438 (Fed Cir. 1991). When "the motivation to combine the teachings of the references is not immediately apparent, it is the duty of the examiner to explain why the combination of the teachings is proper." MPEP § 2142. Moreover, the Federal Circuit Court has "consistently held that 'obvious to try' is not to be equated with obviousness under 35 U.S.C. § 103." *Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720, 725 (Fed. Cir 1990); *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988).

With regard to the obviousness rejections that rely on Rosenthal, Fuller, Labeit, or Minami, it appears that the Examiner, without instruction from the prior art, assumed that the skilled artisan would follow the same inventive path as Applicants. The Rosenthal, Fuller, Labeit, and Minami references all describe methods of DNA sequencing. They do not teach or suggest, alone or in combination with the other cited references, Applicants' claimed invention. "It is impermissible to use the claimed invention as an instruction manual or 'template' to pieced together the teachings of the prior art so that the claimed invention is rendered obvious." In re Fritch, 972 F.2d 1260, 1266 (Fed. Cir. 1992). Stated another way, "[o]ne cannot use hindsight

reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." In re Fine, 837 F.2d 1071, 1075 (Fed. Cir. 1988).

### 2. *Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 Are Patentable Over Stemmer*

Claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 stand rejected under § 102(e) as anticipated by Stemmer (USPN 6,506,603). Applicants respectfully traverse.

The Action argues that it would have been obvious to one skilled in the art to have used dideoxynucleotides, since dideoxynucleotides are nucleotide analogs and are used in the art interchangeably with the recitation of "nucleotide analogs" for accomplishing the same function of terminating extension.

In order to establish a *prima facie* case of obviousness the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP § 2142. The Action failed to establish that Stemmer teaches or suggest all the claim limitations.

The only element that the Action attempts to establish is the "at least one dideoxynucleotide, dideoxynucleotide analog or dideoxynucleotide derivative." However, it would not have been obvious for one of ordinary skill in the art to substitute the "nucleotide analogs" recited in Stemmer's gene shuffling method with a dideoxynucleotide or dideoxynucleotide analog, because, as described above in regard to the anticipation rejection, it appears that such a substitution would not work in Stemmer's method.

Even assuming that Stemmer suggests dideoxynucleotides or dideoxynucleotide analogs, a *prima facie* case for obviousness has not been made because it has not been shown where Stemmer teaches or suggest any of the other elements of the claims. For example, the Action failed to show where Stemmer teaches or suggests elements such as defined primers or annealing

the extended nucleic acid to a second single stranded template whose sequence is not identical to the first template nucleic acid.

In view of the above, Applicants request the withdrawal of the rejection.

### *3. Claim 18 Is Patentable Over Short in View of Rosenthal*

Claim 18 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Short (WO 98/01581) in view of Rosenthal *et al.* (US Patent 6,087,095). Applicants respectfully traverse.

As discussed above, Short does not teach all the claim limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 nor rendered these claims obvious, and therefore it cannot form the basis of the rejection of dependent claim 18 as argued by the Action. Because Short does not teach or suggest all of the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57, viewing Short in light of Rosenthal cannot obviate claim 18, which depends from claim 9.

In addition, there must be some motivation or suggestion to combine the references, which is not present in this case. MPEP § 2143. The goal and purpose of the Rosenthal methods is to create readable sequence data from a single template. There is no suggestion or motivation to combine a reference on DNA sequencing with a method that appears to teach the generation of random mutant polynucleotides. Furthermore, the presently claimed invention recites more than one template wherein the templates are not of identical sequence. It would not be obvious for those of ordinary skill in the art to look to Rosenthal for guidance as the presence of more than one template wherein the templates are not of identical sequence would only confound the sequencing results taught by Rosenthal.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection.

### *4. Claim 18 Is Patentable Over Stemmer in View of Rosenthal*

Claim 18 stands rejected under § 103(a) as unpatentable over Stemmer (USPN 6,506,603) in view of Rosenthal (USPN 6,087,095). Applicants respectfully traverse.

The Action states that it would have been obvious to one of ordinary skill in the art to have modified the method of Stemmer so as to have removed the at least one dideoxynucleotide, dideoxynucleotide analog or dideoxynucleotide derivative as taught by Rosenthal.

As discussed above, the Action failed to establish that Stemmer teaches or suggests all the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57. Because Stemmer does not teach or suggest all of the limitations of these claims, viewing Stemmer in light of Rosenthal cannot obviate claim 18, which depends from claim 9.

In addition, there must be some motivation or suggestion to combine the references, which is not present in this case. MPEP § 2143. The goal and purpose of the Rosenthal methods is to create readable sequence data from a single template. There is no suggestion or motivation to combine a reference on DNA sequencing with a method that appears to teach gene shuffling. Furthermore, the presently claimed invention recites more than one template wherein the templates are not of identical sequence. It would not be obvious for those of ordinary skill in the art to look to Rosenthal for guidance as the presence of more than one template wherein the templates are not of identical sequence would only confound the sequencing results taught by Rosenthal.

In view of the above, Applicants request the withdrawal of the rejection.

### *5. Claim 42 Is Patentable Over Short in View of Fuller*

Claim 42 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Short (WO 98/01581) in view of Fuller (USPN 5,741,676). Applicants respectfully traverse.

The Action states that Fuller teaches the use of alkaline phosphatase and exonuclease, following an amplification reaction, is advantageous because it degrades undesirable primers and NTPs prior to further analysis. The Action states further that one of ordinary skill in the art would have been motivated to modify the method of Short in view of the method of Fuller in order to achieve the benefit of degrading undesirable primer and NTPs.

As discussed above, Short does not teach all the claim limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57 nor rendered these claims obvious, and therefore it cannot form the basis of the rejection of dependent claim 42 as argued by the Action. Because Short does not teach or suggest all of the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57, viewing Short in light of Fuller cannot obviate claim 42.

Furthermore, claim 42 reads, "The method of claim 40, wherein said length-altering agent further comprises treatment with alkaline phosphatase and an exonucleoase." As recited in the claim, alkaline phosphatase and an exonucleoase are themselves length-altering agents. The Action states that "[o]ne of ordinary skill in the art would have been motivated to modify the method of Short in order to have achieved the benefit of degrading undesirable primer and NTPs, enhancing further analysis." Degrading undesirable primers and NTPs are not elements of claim 42. Thus, it has not been established that one of ordinary skill in the art would have been motivated to combine Short and Fuller to make the invention recited in claim 42.

In view of the above, Applicants request the withdrawal of the rejection.

### *6. Claim 42 Is Patentable Over Volkov in View of Fuller*

Claim 42 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Volkov (USPN 6,534,292) in view of Fuller (USPN 5,741,676). Applicants respectfully traverse.

As discussed above, Volkov does not teach all of the elements of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46-47, 53-55, and 57 nor rendered these claims obvious, and therefore it cannot form the basis of the rejection of dependent claim 42 as argued by the Action.

Furthermore, the Rule 131 Declaration of George L. Murphy, Robert A. Setterquist, and Andrew D. Ellington establishes that Volkov is not prior art. Applicants, therefore, request the withdrawal of this rejection.

### *7. Claim 42 Is Patentable Over Stemmer in View of Fuller*

Claim 42 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Stemmer (USPN 6,506,603) in view of Fuller (USPN 5,741,676). Applicants respectfully traverse.

The Action states that it would have been obvious to one of ordinary skill in the art to modify the method of Stemmer by adding alkaline phosphatase and exonuclease following the addition of a length-altering agent as described in Fuller.

As discussed above, the Action failed to establish that Stemmer teaches or suggests all the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57. Because Stemmer does not teach or suggest these limitations, viewing Stemmer in light of Fuller cannot obviate claim 42.

Also as discussed above, claim 42 reads, "The method of claim 40, wherein said length-altering agent further comprises treatment with alkaline phosphatase and an exonucleoase." As recited in the claim, alkaline phosphatase and an exonucleoase are themselves length-altering agents. The Actions states that "[o]ne of ordinary skill in the art would have been motivated to modify the method of Stemmer in order to have achieved the benefit of degrading undesirable primer and NTPs, enhancing further analysis." Degrading undesirable primers and NTPs are not elements of claim 42. Thus, it has not been established that it would have been obvious to one of ordinary skill in the art to combine Stemmer and Fuller to arrive at the invention claimed 42.

In view of the above, Applicants request the withdrawal of the rejection.

### *8. Claim 45 Is Patentable Over Short in View of Labeit*

Claim 45 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Short (WO 98/01581) in view of Labeit (DNA (1986) 5(2): 173-177). Applicants respectfully traverse.

The Action states that, in view of the teachings of Labeit, it would have been obvious to one of ordinary skill in the art to have modified the method of Short so as to have used an $\alpha$-phosphorothioate nucleotide, "in order to have achieved the benefit of providing a more efficient and more effective means of obtaining sequence information."

Claim 45 is not directed to a method of obtaining sequence information. Thus, the Action failed to establish a motivation or suggestion to combine the references.

Furthermore, as discussed above, Short does not teach or suggest all the claim limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57, and therefore it cannot form the basis of the rejection of dependent claim 45 as argued by the Action.

In view of the above, Applicants request the withdrawal of the rejection.

### *9. Claim 45 Is Patentable Over Volkov in View of Labeit*

Claim 45 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Volkov (USPN 6,534,292) in view of Labeit (DNA (1986) 5(2): 173-177). Applicants respectfully traverse.

As discussed above, Volkov does not teach all of the elements of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46-47, 53-55, and 57 nor rendered these claims obvious, and therefore it cannot form the basis of the rejection of dependent claim 45 as argued by the Action.

Furthermore, the Rule 131 Declaration of George L. Murphy, Robert A. Setterquist, and Andrew D. Ellington establishes that Volkov is not prior art. Applicants, therefore, request the withdrawal of this rejection.

### *10. Claim 45 Is Patentable Over Stemmer in View of Labeit*

Claim 45 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Stemmer (USPN 6,506,603) in view of Labeit (DNA (1986) 5(2): 173-177). Applicants respectfully traverse.

The Action states that, in view of the teachings of Labeit, it would have been obvious to one of ordinary skill in the art to have modified the method of Stemmer so as to have used an $\alpha$-phosphorothioate nucleotide, "in order to have achieved the benefit of providing a more efficient and more effective means of obtaining sequence information."

Claim 45 is not directed to a method of obtaining sequence information. Thus, the Action failed to establish a motivation or suggestion to combine the references.

Furthermore, as discussed above, the Action failed to establish that Stemmer teaches or suggests all the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57. Because Stemmer does not teach or suggest these limitations, viewing Stemmer in light of Fuller cannot obviate claim 45.

In view of the above, Applicants request the withdrawal of the rejection.

### *11. Claims 47-48 and 56 Are Patentable Over Short in View of Minami*

Claims 47-48 and 56 stand rejection under 35 U.S.C. § 103(a) as being unpatentable over Short (WO 98/01581) in view of Minami (USPN 5,106,585). Applicants respectfully traverse.

The Action states that Minami teaches the advantages of using the Maxam-Gilbert treatment for creating fragments. The Action states further that, in view of the teachings of Minami, it would have been obvious to one of ordinary skill in the art to modify the method of Short so as to have used the Maxam-Gilbert chemical treatment for producing nucleic acid fragments.

As discussed above, Short does not teach or suggest all the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57, and therefore it cannot form the basis of the rejection of dependent claims 47-48 and 56 as argued by the Action.

Furthermore, there is no motivation or suggestion to combine Short and Minami. The Action quotes Minami as saying that "Maxam Gilbert is being widely used because it has the advantages of involving relatively simple experimental operations and comparing favorably in rapidity and accuracy with other determination procedures." The Action cites this statement as the motivation for combining the teachings of Minami with those of Short. However, the benefits stated by Minami over other "determination procedures" refers specifically to procedures for determining the base sequence of DNA (see Minami, col. 1, lines 21-23). The instant claims are not directed to methods for sequencing DNA. Therefore, alleged benefits over other sequencing methods does not provide the requisite motivation or suggestion to combine the references.

In view of the foregoing, Applicants request the withdrawal of the rejection.

***12. Claims 48 and 56 Are Patentable Over Volkov in View of Minami***

Claims 48 and 56 stand rejection under 35 U.S.C. § 103(a) as being unpatentable over Volkov (USPN 6,534,292) in view of Minami (USPN 5,106,585). Applicants respectfully traverse.

As discussed above, Volkov does not teach all of the elements of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46-47, 53-55, and 57 nor rendered these claims obvious, and therefore it cannot form the basis of the rejection of dependent claims 48 and 56 as argued by the Action.

Furthermore, the Rule 131 Declaration of George L. Murphy, Robert A. Setterquist, and Andrew D. Ellington establishes that Volkov is not prior art. Applicants, therefore, request the withdrawal of this rejection.

### *13. Claims 47-48 and 56 Are Patentable Over Stemmer in View of Minami*

Claims 47-48 and 56 stand rejection under 35 U.S.C. § 103(a) as being unpatentable over Stemmer (USPN 6,506,603) in view of Minami (USPN 5,106,585). Applicants respectfully traverse.

The Action states that Minami teaches the advantages of using the Maxam-Gilbert treatment for creating fragments. The Action states further that, in view of the teachings of Minami, it would have been obvious to one of ordinary skill in the art to modify the method of Stemmer so as to have used the Maxam-Gilbert chemical treatment for producing nucleic acid fragments.

As discussed above, the Action failed to establish that Stemmer teaches or suggests all the limitations of claims 1-7, 9, 11-17, 19, 21-41, 43-44, 46, 53-55, and 57. Because Stemmer does not teach or suggest these limitations, viewing Stemmer in light of Minami cannot obviate claims 47-48 and 56.

Furthermore, there is no motivation or suggestion to combine Stemmer and Minami. The Action quotes Minami as saying that "Maxam Gilbert is being widely used because it has the advantages of involving relatively simple experimental operations and comparing favorably in rapidity and accuracy with other determination procedures." The Action cites this statement as the motivation for combining the teachings of Minami with those of Stemmer. However, the benefits stated by Minami over other "determination procedures" refers specifically to procedures for determining the base sequence of DNA (see Minami, col. 1, lines 21-23). The instant claims are not directed to methods for sequencing DNA. Therefore, alleged benefits over other sequencing methods does not provide the requisite motivation or suggestion to combine the references.

In view of the foregoing, Applicants request the withdrawal of the rejection.

## E. Summary

In light of the preceding remarks, Applicants respectfully submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. Should Examiner Spiegler have any questions regarding this response, please contact the undersigned at the telephone number listed below.

## REQUEST FOR EXTENSION OF TIME

Pursuant to 37 C.F.R. § 1.136(a), Applicants petition for an extension of time of one month to and including June 25, 2004 in which to respond to the Office Action dated February 25, 2004.

Pursuant to 37 C.F.R. § 1.17, a check in the amount of $55.00 is enclosed, which is the process fee ($55.00) for a one-month extension of time.

If the check is inadvertently omitted, or should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, or should an overpayment be included herein, the Commissioner is authorized to deduct or credit said fees from or to Fulbright & Jaworski L.L.P. Account No.: 50-1212/AMBI:055/MBW.

Respectfully submitted,

GINA SHISHIMA Reg. No. 45,104
FOR Mark B. Wilson
Reg. No. 32,759
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3035

Date: June 25, 2004

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
George L. Murphy et al.

Serial No.: 09/613,535

Filed: July 10, 2000

For: METHODS FOR RECOMBINATORIAL NUCLEIC ACID SYNTHESIS

Group Art Unit: 1637

Examiner: Spiegler, A.H.

Atty. Dkt. No.: AMBI:055US

CERTIFICATE OF MAILING
37 C.F.R 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

6/25/04
Date

Mark B. Wilson GINA SMITH

**DECLARATION OF GEORGE L. MURPHY, ROBERT A. SETTERQUIST, AND ANDREW D. ELLINGTON UNDER 37 C.F.R. § 1.131**

We, George L. Murphy, Robert A. Setterquist, and Andrew D. Ellington, hereby declare as follows:

1. We are the inventors of the subject matter of all claims currently pending in the referenced patent application.

2. We understand that the Patent and Trademark Examiner found the claimed subject matter of the referenced application to be anticipated by U.S. Patent 6,534,292.

3. We are submitting this Declaration to set forth evidence that we invented the subject matter of the claimed invention prior to May 8, 2000, the priority date of U.S. Patent 6,534,292.

4. As evidence of our conception, we attach, as Exhibit 1, pages describing all technological aspects of the invention from an invention disclosure made following Ambion, Inc. standard procedures prior to May 8, 2000.

5. The attached invention disclosure describes, for example, annealing a defined primer nucleic acid to a first single stranded nucleic acid and performing a first extension wherein a dideoxynucleotide is incorporated into the extension product (see Step 5). Step 6 describes removing the dideoxynucleotide from the extended nucleic acid. Step 7 describes denaturing the extended nucleic acid from the first template nucleic acid, annealing the extended nucleic acid to a second template nucleic acid whose sequence is not identical to the first template nucleic acid, and performing a second extension wherein a dideoxynucleotide is incorporated into the twice extended nucleic acid. Step 7 also provides that Steps 6 and 7 can be repeated a desired number of times.

6. We were diligent in reducing the claimed invention to practice from the time of the invention disclosure up to and including the July 10, 2000 filing date of the instant patent application.

7. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date: 6/12/04

George L. Murphy

Date: _____

Robert A. Setterquist

Date: _____

Andrew D. Ellington

5. The attached invention disclosure describes, for example, annealing a defined primer nucleic acid to a first single stranded nucleic acid and performing a first extension wherein a dideoxynucleotide is incorporated into the extension product (see Step 5). Step 6 describes removing the dideoxynucleotide from the extended nucleic acid. Step 7 describes denaturing the extended nucleic acid from the first template nucleic acid, annealing the extended nucleic acid to a second template nucleic acid whose sequence is not identical to the first template nucleic acid, and performing a second extension wherein a dideoxynucleotide is incorporated into the twice extended nucleic acid. Step 7 also provides that Steps 6 and 7 can be repeated a desired number of times.

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Date: _____

_____
George L. Murphy

Date: _____

_____
Robert A. Setterquist

Date: 6/22/2004

Andrew D. Ellington
Andrew D. Ellington

5. The attached invention disclosure describes, for example, annealing a defined primer nucleic acid to a first single stranded nucleic acid and performing a first extension wherein a dideoxynucleotide is incorporated into the extension product (see Step 5). Step 6 describes removing the dideoxynucleotide from the extended nucleic acid. Step 7 describes denaturing the extended nucleic acid from the first template nucleic acid, annealing the extended nucleic acid to a second template nucleic acid whose sequence is not identical to the first template nucleic acid, and performing a second extension wherein a dideoxynucleotide is incorporated into the twice extended nucleic acid. Step 7 also provides that Steps 6 and 7 can be repeated a desired number of times.

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Date: _____

George L. Murphy

Date: 6-24-04

Robert A. Setterquist

Date: _____

Andrew D. Ellington

**Example 1.** The rEVOLUTION process is described below, using linear DNA, for the *in vitro* recombination of a multiple gene pool.

**Step 1:** Multiple gene pool: Amplify the genes or gene fragments of interest attaching "linker" segments to the 5' and 3' ends. Alternatively, the gene(s) can be cloned and then excised from a plasmid vector with the 5' and 3' "linker" segments attached (The linker segments are not part of the gene but will serve to simplify subsequent steps and a final optional PCR). The DNA will be linear and double stranded at this point. The 5' linker has a site for a restriction enzyme (RE) that does not cut within the gene and that generates a 5' overhang.

**Step 2:** Digest the double stranded DNA with RE to generate the 5' overhang at the "5' end" of the genes.

**Step 3:** Resistant adapter ligation: Ligate to the 5' end an adapter oligonucleotide with numerous 3' phosphorothioate nucleotides (or some other modification, .e.g. 3' phosphate / or attach this strand to a matrix-e.g. biotin/SA magnetic bead) to render it resistant to nuclease attack. The 3' end of the lower "non-coding" strand must be resistant to 3'-5' exonuclease treatment.

**Step 4:** Single strand (ss) polynucleotide production: Exonuclease III treat the sample to degrade the upper "coding" strand. (Alternatively, we could do a lambda exonuclease treatment if we synthesized the DNA with a correctly phopshorylated/ non-phosphorylated set of PCR primers in Step 1.) This generates a group of ssDNAs - the "non-coding" strand, blocked at the 3' ends, with "linker" sequences on the ends.

**Step 5:** Polymerase extension with dideoxynucleotides: Synthesize an oligonucleotide complementary to the 5' "linker" (all or part) with or without some chosen number of nucleotides within the 5' end of the gene(s). This oligonucleotide will serve as a primer in a "dideoxy sequencing type reaction". The dideoxy reactions for each gene template can be performed individually or in combination in a single tube, avoiding the use of excess primer. The reaction uses Sequenase or exo$^-$ Klenow (or a mixture of these and/or T7 polymerase). The use of a thermostable polymerase here may be advantageous so as to avoid multiple additions. This reaction generates a collection of ssDNAs that represent single nucleotide extensions of a given gene. These have dideoxynucleotides incorporated at their 3' termini. The "non-coding" template DNAs are still intact.

**Step 6:** Exonuclease III: Add exonuclease III and perform a limited digest so as to remove the terminal dideoxy nucleotides (we believe that Exo III will do this; can we use something else?). Heat to stop the reaction and kill Exo III (heat should kill Exo III; another method?).

**Step 6a:** Primer/nucleotide removal: (This is called 6a, because it is performed only after the first and last rounds.) Pass the mix over a size exclusion column to remove excess primer. This will also remove dideoxy- and deoxynucleotides.

**Step 7:** Denature/anneal/extend: Heat to denature, snap freeze, anneal at low T. (This allows for cross-strand annealing among different genes.) Perform a polymerase reaction again as in Step 5, but without added primer. The nested set of single strand fragments created in Steps 5 and 6 will serve to prime here.

Go to Step 6: Go to Step 7:

**Step 8:** Final fill-in reaction: At end of desired number of cycles, repeat Step 6, Step 6a, and Step 7. However, don't include dideoxynucleotides. Just fill in the sense strand using Sequenase (exo$^-$ Klenow) and deoxynucleotides. After that, add the 3' end oligo and make antisense strands, so everything is double-stranded.

**OPTIONAL Step 9:** PCR amplify using, as primers, the linker sequences mentioned above.

**Step 10:** Clone fragments into a suitable vector and transform *E. coli* or another organism to make a library of clones for expression of the recombined genes or gene fragments.

**Example 2.** The process may also use single stranded circular DNA as described below.

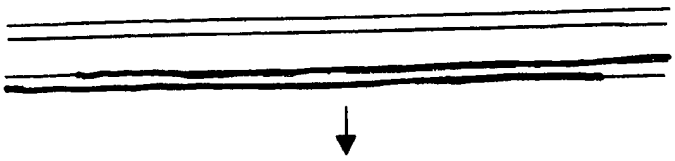
**Step 1:** Multiple gene pool: Clone the genes or gene fragments of interest, individually, into a vector from which single strand DNA can be prepared such as M13mp18. The genes/fragments can be cloned in either orientation.

**Step 2:** Single strand polynucleotide production: From each clone, prepare single strand circular M13 DNA with the gene or fragment of interest. This generates a group of ss circular DNAs that are resistant to Exonuclease III attack.

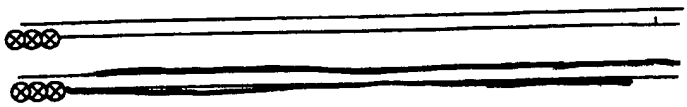
The remaining steps are identical to those described in Example 1. However, it is unnecessary to insert into a vector. The last step will generate double stranded M13s with the recombined genes or gene fragments and will be suitable for cloning directly.

↑ EVOLUTION
(1)

STEP 1
PCR AMPLIFY ALL GENES W/ LINKERS

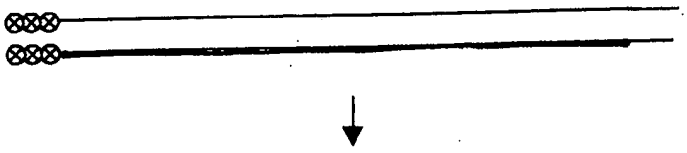


STEPS 2/3
ATTACH MODIFIED OLIGOS @ VIA Adapter OR BIND TO MATRIX VIA ADAPTER OLIGOS

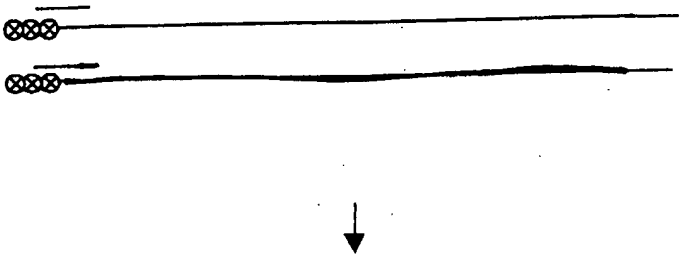


Exo 3

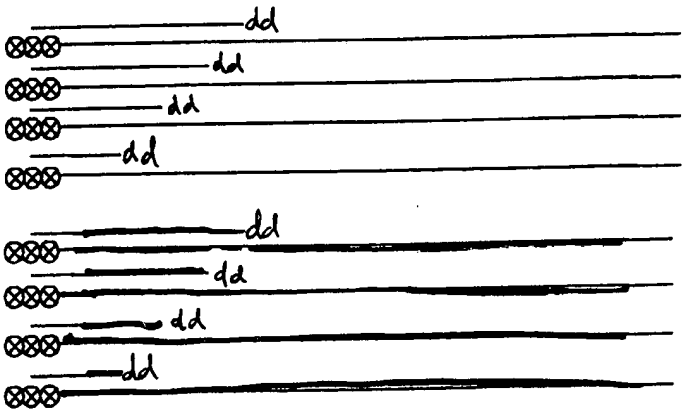
STEP 4
EXONUCLEASE SENSE STRAND



STEP 5
ANNEAL PRIMER OLIGO – PART LINKER PART GENE



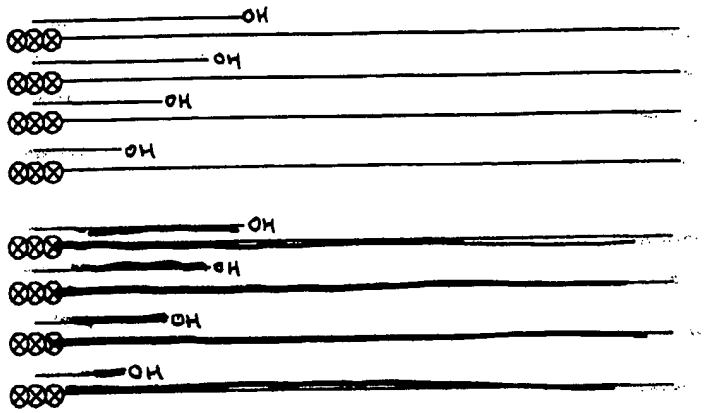
DIDEOXY + DEOXY "SEQUENCING" REACTION




MAY want to do this separately for each individual gene – up to AND THRU STEP 5 AND MAYBE STEP 6 LIKELY

STEP 6

limited Exo3 to remove dd




6a

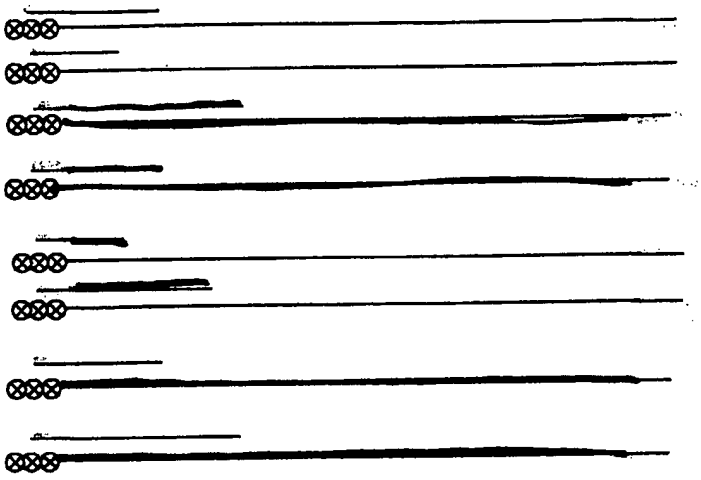
Pass mix over size exclusion column to eliminate excess primer (+ dideoxy + deoxynucleotides)

performed only after 1st And last rounds

Kill Exo3 Δ?

STEP 7

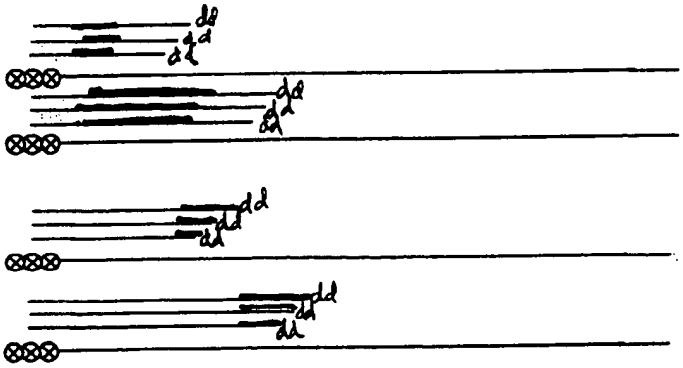
Δ kill + denature snap freeze Anneal lowT



dideoxy / deoxy "sequencing rxn"

Generate hybrids

not all shown




GO TO STEP 6, REPEAT DESIRED # OF TIMES
AFTER LAST DO 6, 6A, 7
SYNTHESIZE OPPOSITE STRAND – LIBRARY